

Full Length Research Paper

Chemical composition and *in vitro* antioxidant, cytotoxicity activities of *Zingiber officinale* Roscoe essential oil

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This study was designed to examine the *in vitro* antioxidant and cytotoxicity activities of *Zingiber officinale* Roscoe (ginger) rhizome essential oil. GC-MS analysis of the essential oil resulted in the identification of 7 compounds, representing 90.06% of the oil, the major constituents were described as α -zingiberene (52.35%), β -pinene (14.20%) and β -sesquiphellandrene (12.11%). The oil was subjected to screening for their possible antioxidant activity by means of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and β -carotene bleaching test. In the DPPH test system, the concentrations providing 50% inhibition (IC₅₀) values of free radical-scavenging activity of ginger essential oil was determined to be 0.0144% (v/v). In the β -carotene bleaching test system, we tested series concentration of samples to show the antioxidant activities of the oil, the IC₅₀ values of ginger essential oil was 0.55% (v/v). Ginger essential oil exhibited the strong cytotoxicity towards two human cancer cells at low concentration. Its IC₅₀ values on HO-8910 and Bel-7402 were 0.00643 and 0.00256% (v/v), respectively.

Key words: *Zingiber officinale* Roscoe, essential oil, GC-MS, antioxidant activity, cytotoxicity activity.

INTRODUCTION

It is well known that reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species (Nain et al., 2011). Reactive oxygen species, causing damage to DNA, proteins and lipids, have been associated with carcinogenesis, coronary heart disease, and many other health problems (Sasidharan and Menon, 2010). For this reason, anti-oxidation is an extremely significant activity which can be used as a preventive agent against diseases (Motawi et al., 2011). The possible ways to fight these diseases is to improve our body's transformation due to antioxidant defenses. High consumption of plants, fruits and vegetables has been associated with a lowered incidence of such diseases (Bajpai et al., 2009).

Cancer is now a major cause of death in the world. Synthetic drugs are often the only option for cancer chemotherapy (Chabner and Roberts, 2005; DeVita and Chu, 2008; Ma and Wang, 2009). However, most synthetic drugs kill not only tumor cells, but also normal cells, and most have severe side effects (Cragg et al., 2009). Therefore, there is an urgent need for novel treatment options with improved features. Recently, natural products from medicinal plants represent a fertile ground for the development of novel anticancer agents (Zu et al., 2010).

During recent years, essential oils and their constituents, products from secondary metabolism of plants, have many applications in ethnomedicine. These oils have been widely used in the pharmaceutical, cosmetic, and food and beverage industries (Nogueira et al., 2011). Biological activity of essential oils depends on their composition. These oils are natural mixtures of terpenes obtained from aromatic and pharmaceutical plants, mainly monoterpenes and sesquiterpenes (Daferera et al., 2002).

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Thus the importance of conducting studies on essential oils, lies not only in the chemical characterization but also in the possibility of linking the chemical contents with particular functional properties. In this regard, it is advisable to assess their biological activities that not only highlight aromatic or preservative activities but also correlate with functional properties potentially useful for pharmaceuticals, nutraceuticals and cosmetic applications. Following this idea, we assessed the chemistry composition and the antioxidant, cytotoxicity activities of ginger essential oil.

Ginger is one of the oldest herbs known by the people and is one of the earliest spices to be known in the east. Ginger of the commerce consists of thick scaly rhizomes of the plant *Zingiber officinale*, belonging to the family *Zingiberaceae*. The rhizomes (spice of commerce) are aromatic, thick lobed, branched and scaly structures with a spicy lemon-like scent. Ginger has a long history of medicinal use in Asian countries for the treatment of asthma, common cold disorders, nervous disease, stroke, toothache, gastrointestinal constipation, inflammation, migraine, oxidant stress, hypercholesterolaemia, helminthiasis and schistosomiasis (Langmead and Rampton, 2001; Sekiya et al., 2004; Iqbal et al., 2006; Ali et al., 2008; Chohan et al., 2008; Ghayur et al., 2008; Islam and Choi, 2008; Lin et al., 2010; Iwami et al., 2011). Many pharmacological properties of ginger have been identified, including essential oil, zingiberol, zingiberone, zingiberene, pungent and non-pungent components such as shogaol, gingerol, and zingerone, are included in ginger (Govindarajan, 1982). The chemical composition of ginger are different due to different geographical locations (Bartley and Foley, 1994; Bailey-Shaw et al., 2008; Macleod and Pieris, 1984; Vanbeek et al., 1987), extraction methods (Chen and Ho, 1988; Badalyan et al., 1998; Zancan et al., 2002; Shao et al., 2003; Gong et al., 2004; Singh et al., 2008) and processing technologies (Bartley and Jacobs, 2000; Mishra et al., 2004; Variyar et al., 2006; Ding et al., 2011). Several lines of evidence indicate that the components are dominantly related to the actions of ginger (Mascolo et al., 1989).

In this paper, we report the results of a study aimed to identify the composition and evaluate the *in vitro* antioxidant activities and toxicology properties of *Zingiber officinale* Roscoe rhizome essential oil and provide scientific foundation for further exploitation.

MATERIALS AND METHODS

Essential oils of ginger (*Zingiber officinale* Roscoe) was obtained from a commercial source (Xiamen Denyla Essential Oil Co., Ltd., Xiamen, China).

Gas chromatography-mass spectrum

The analysis of the essential oil was performed using a VG platform GC-MS system equipped with a DB-5MS capillary column (30 × 0.25 mm i.d.; film thickness 0.25 µm). For GC-MS detection, an

electron ionisation system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and detector MS transfer line temperatures were set at 160 and 265°C, respectively. Column temperature was initially kept at 60°C and held for 2 min, then gradually increased to 160°C at 3°C/min rate, held for 4 min and finally raised to 220 at 6°C/min. 0.1 µl essential oil were injected manually. The components were identified by NIST library data of the GC-MS system and literatures.

Antioxidant activity

Free radical-scavenging activity: DPPH assay

The stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a reagent in this spectrophotometric assay (Amarowicz et al., 2004). 100 µl serial concentrations of the sample (0.00305 to 0.195%) was mixed with 1.4 ml of ethanol and then added to 1 ml 0.004% (W/V) DPPH (Sigma-Aldrich) in ethanol. The mixture was shaken vigorously and then immediately placed in a spectrophotometer to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. Ascorbic acid (Sigma), a stable antioxidant, was used as a synthetic reference. The radical-scavenging activities of samples, expressed as percentage inhibition of DPPH, were calculated according to the formula: Inhibition percentage (Ip) = [(AB-AA)/AB] · 100 (Yen and Duh, 1994) where AB and AA are the absorbance values of the blank sample and of the tested samples checked after 70 min, respectively. Samples were analyzed in triplicate.

Antioxidant activity: β-carotene bleaching test

Antioxidant activity of the samples was determined using β-carotene bleaching test (Taga et al., 1984). Approximately 10 mg of β-carotene (type I synthetic, Sigma-Aldrich) was dissolved in 10 ml chloroform. The carotene-chloroform solution, 0.2 ml, was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma-Aldrich) and 200 mg Tween 40 (Sigma-Aldrich). Chloroform was removed using a rotary evaporator (RE-52AA) at 40°C for 5 min, and to the residue, 50 ml of distilled water was added, slowly with vigorous agitation, to form an emulsion. 5 ml of the emulsion were added to a tube containing 0.2 ml of the samples solution and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Control samples contained 10 µl of water instead. Butylated hydroxytoluene (BHT; Sigma-Aldrich), a stable antioxidant, was used as a synthetic reference. The antioxidant activity was expressed as inhibition percentage with reference to the control after a 60 min incubation using the following equation:

$$AA = 100(DR_C - DR_S)/DR_C,$$

where AA = antioxidant activity; DR_C = degradation rate of the control = [ln(a/b)/60]; DR_S = degradation rate in presence of the sample = [ln(a/b)/60]; a = absorbance at time 0; b = absorbance at 60 min. Samples were analyzed in triplicate.

Cytotoxicity assay

Maintenance of human cancer cell lines

Human ovarian cancer cell lines HO-8910 and human hepatocellular liver carcinoma cell line Bel-7402 were purchased from China Center for Type Culture Collection (Wuhan, China). These cell lines

Table 1. Composition percentage of ginger essential oil.

No.	R.T.(min)	Compounds	m/z	%
1	4.71	α -Pinene	136	2.14
2	5.10	Santolina triene	136	6.06
3	7.61	β -Pinene	136	14.20
4	22.35	Copaene	204	0.491
5	27.23	α -Curcumene	202	2.71
6	27.8	Zingiberene	204	52.35
7	28.33	β -Sesquiphellandrene	204	12.11
Total				90.06

were grown and maintained in a humidified incubator at 37°C with a 5% CO₂ atmosphere. Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin was used for the cell cultures.

Cytotoxicity assay

The cytotoxic effects of the ginger essential oil on two human tumor cell lines were assayed by the MTT assay (Mosmann, 1983). The cells were seeded at a density of 5×10^4 cells/well. The essential oils were serially double diluted from 0.01% to 0.000625% (v/v), and 200 µl liquid of each concentration was applied to the wells of a 96-well plate containing confluent cell monolayers (six wells per concentration). The dilution medium without the sample served as a control. After 48 h of incubation, MTT solution (5 mg/ml) was then added to each well, and the formazan precipitate was dissolved in 200 µL dimethyl sulfoxide after 4 h incubation. The content of the wells was homogenized on a microplate shaker for 5 min. The optical densities (OD) were measured on a microplate ELISA reader at 492 nm. All tests and analyses were run in triplicate and mean values were recorded. The cell survival curves were calculated after comparing with the control. The percentage viability was calculated as follows:

$$\% \text{viability} = \frac{\text{mean absorbance of treated wells}}{\text{mean absorbance of untreated wells (no oil)}} \times 100$$

Values are expressed as means \pm SD of three independent experiments.

RESULTS AND DISCUSSION

Chemical composition

Govindarajan (1982) reported the volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingiberene (35%), curcumene (18%) and farnesene (10%), with lesser amounts of bisabolene and β -sesquiphellandrene. A smaller percentage of at least 40 different monoterpenoid hydrocarbons are present with 1, 8-cineole, linalool, borneol, neral, and geraniol being the most abundant. Many of these volatile oil constituents contribute to the distinct aroma and taste of ginger. Zhou et al. (1998) identified 66 compounds in the essential oil of ginger, of which the major compounds are camphene, β -phellandrene and 1, 8-cineol. Other constituents include

α -zingiberen, β -bisabolen, (+)-ar-curcumen, β -sesquiphellandren and acyclic afarnesen. Singh et al. (2008) reported the main components of ginger essential oil obtained by hydrodistillation were geranial (25.9%), α -zingiberen (9.5%), farnesene (76%) and β -sesquiphellandrene (5.1%). In our study, 7 components were identified in the *Zingiber officinale* essential oil (Table 1), representing 90.06% of the oil. The major constituents of the oil were described as α -zingiberene (52.35%), β -pinene (14.20%), β -sesquiphellandrene (12.11%), santolina triene (6.06%), α -curcumene (2.71%) and α -pinene (2.14%), respectively.

Antioxidant activity

The antioxidant activities of the ginger essential oil studied here were determined by two complementary test systems, namely DPPH assay and β -carotene bleaching test.

Free radical-scavenging activity: DPPH assay

In the DPPH test system, at a concentration of 0.195%, free radical scavenging activity of ginger essential oil was determined to be $85.19\% \pm 7.46$, whereas at a concentration of 0.0016% was $32.52\% \pm 6.17$ (Figure 1). The concentrations providing 50% inhibition (IC₅₀) values of ginger essential oil was 0.0144 (v/v) that is 0.0122 mg/ml. The antioxidant activity of the ginger essential oil was mostly related to their concentrations. When compared to ascorbic acid (IC₅₀ = 1.05 mg/ml \pm 0.39, figure not shown), the ginger essential oil showed much stronger free radical scavenging activity. In general, the free radical scavenging activity of ginger essential oil was very excellent.

β -Carotene bleaching test

We assessed the lipid peroxidation inhibitory activity of ginger essential oil by the β -carotene bleaching test (Figure 2). Results were consistent with data obtained from the DPPH test. At a concentration of 10%, the

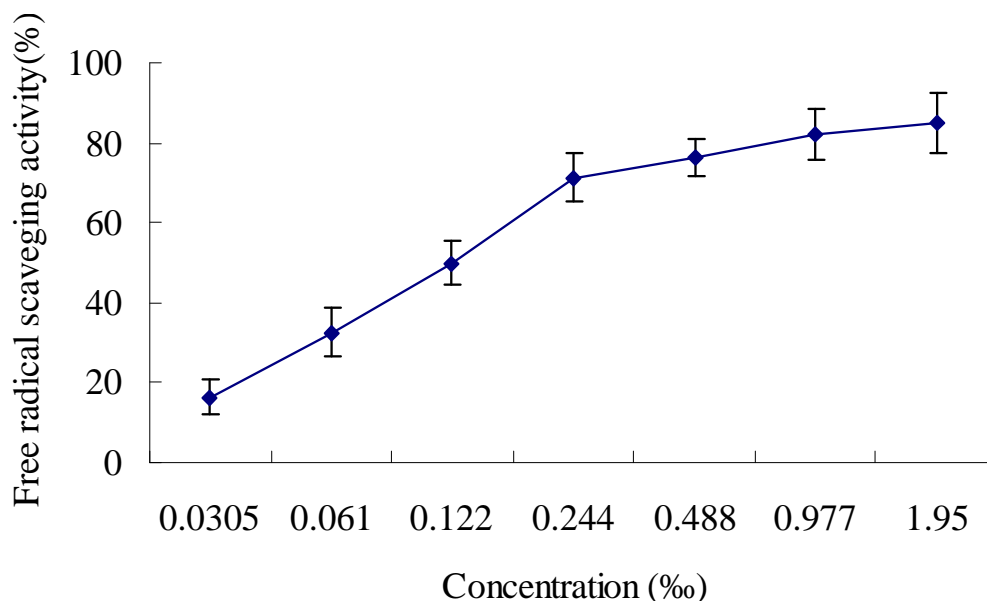


Figure 1. Free radical-scavenging activity of ginger essential oil using DPPH assays.

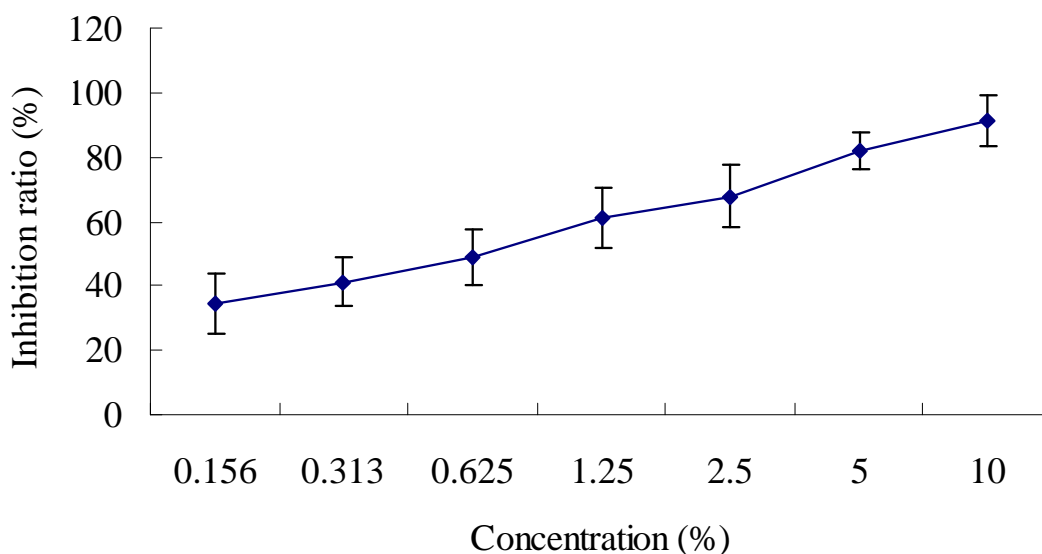


Figure 2. Antioxidant activity percentage of ginger essential oil determined by β -carotene bleaching test.

bleached β -carotene abilities was $91.43\% \pm 7.93$, whereas at the concentration of 5 to 0.156% the bleached β -carotene abilities were in the range of $81.86\% \pm 5.83$ – $34.34\% \pm 9.24$. The concentrations providing 50% inhibition (IC_{50}) values of ginger essential oil was 0.55%, that is 0.468 mg/ml. When compared to butylated hydroxytoluene, ($IC_{50} = 1.74$ mg/ml ± 0.48 , figure not shown), the ginger essential oil was much more effective.

Sacchetti et al. (2005) compared the antioxidants, antiradicals and antimicrobials 11 essential oils. Ginger essential oil exhibited moderate antioxidant activity, with

free radical scavenging activity more than 50% in DPPH assay and antioxidant activity more than 60% in β -carotene bleaching test. This is for the difference in the essential oil composition and test method.

Cytotoxic activity towards cancer cells

In our study, to investigate the cytotoxic activities, two human tumor cell lines, HO-8910 and Bel-7402 were exposed to increasing concentrations of ginger essential

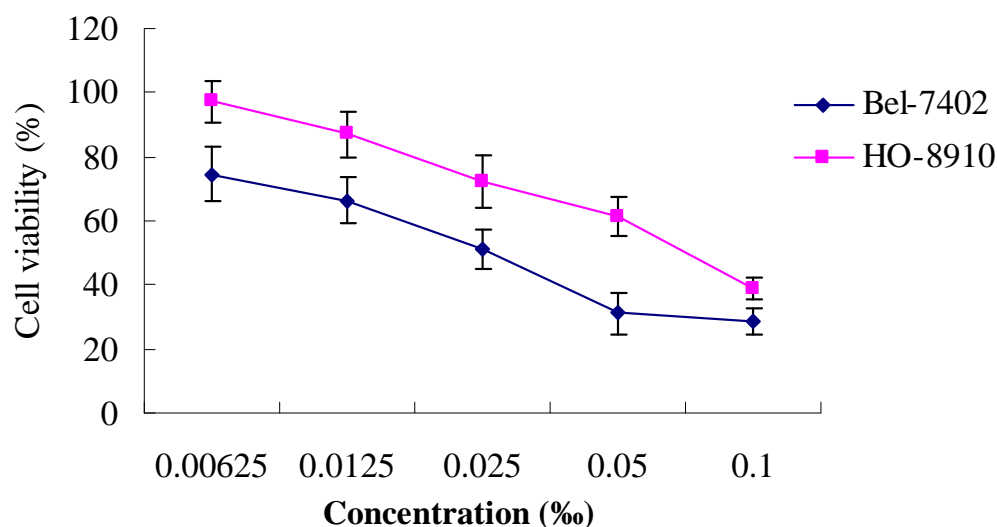


Figure 3. Dose-dependent cytotoxicity of ginger essential oil (48 h exposure) towards HO-8910 and Bel-7402 cell lines as determined by the MTT assay. Values are expressed as means \pm SD of three independent experiments. Standard deviations were less than 10%.

oil. Cell viability was determined by the MTT assay. As shown in Figure 3, the ginger essential oil revealed different cytotoxic activities towards the two human cancer cell lines investigated. In general, a dose-dependent decrease in the survival of the two tumor cell lines was observed. At a concentration of 0.000625% (v/v), the cell viability treated by ginger essential oil for HO-89810 and Bel-7402 were $97.37\% \pm 3.49$ and $74.51\% \pm 6.46$, respectively. At a concentration of 0.01% (v/v), ginger essential oil exhibited strong cytotoxicities towards the two tumor cell lines, the cell viability for HO-89810 and Bel-7402 were $39.17\% \pm 6.63$ and $28.46\% \pm 8.42$, respectively. The concentrations providing 50% inhibition (IC_{50}) values of ginger essential oil against HO-8910 and Bel-7402 were 0.00547 mg/ml and 0.00218 mg/ml, respectively. Bel-7402 was more sensitive cell line compared to HO-8910. The ginger oil inhibited adducts formation significantly and dose dependently (Hashim et al., 1994). Chrubasik et al. (2006) tested the ability to suppress the formation of DNA adducts by aflatoxin B1 *in vitro* in a microsomal enzyme-mediated reaction of ginger oil. Zu et al. (2010) reported the *in vitro* cytotoxicity toward three human cancer cell lines PC-3, A-549 and MCF-7, the IC_{50} values toward PC-3 and A-549 were 0.077%, 0.107%, but MCF-7 is not sensitive to ginger essential oil. HO-8910 and Bel-7402 in our test were much more sensitive to ginger essential oil compared to PC-3, A-549 and MCF-7.

Conclusion

Ginger essential oil exhibited very excellent free radical scavenging activity at very low concentration, and also

had good lipid peroxidation inhibitory activity by the β -carotene bleaching test. In both test systems, ginger essential oil showed strong antioxidant activity. Furthermore, ginger essential oil exhibited strong cytotoxic activities towards HO-8910 and Bel-7402 at low concentration, and Bel-7402 was more sensitive than HO-8910.

Biological activity of essential oils depends on their composition. These oils are natural mixtures of terpenes obtained from aromatic and pharmaceutical plants, mainly monoterpenes and sesquiterpenes (Daferera et al., 2002). In introduction, it is very difficult to attribute the biological effect of a total essential oil to one or a few active principles, because in addition to the major compounds, also minor compounds may make a significant contribution to the oil's activity. From the results above we could infer that ginger essential oil, indicating the strong antioxidant and cytotoxicity activities, is a very important botanical dietary supplement and may explore its nutraceutical role in human diet.

However, it is commendable that further analysis should be carried out on other food poisoning agents, such as *Listeria* sp., *Salmonella* sp.

Some compounds present in ginger may exert cancer preventive effects by inducing apoptosis in cancerous or transformed cells.

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